AMENDMENTS TO THE SPECIFICATION

On page 1, line 1 of the Specification, please add:

This application is a National Phase Application under § 371 of International Application Number PCT/EP03/07605 filed on July 14, 2003.

Please amend the paragraph at the bottom of page 1 and top of page 2 as follows:

Accordingly, there is an urgent need to make available a vaccine capable of significantly reducing mortalities due to piscirickettsiosis in fish. The present invention is based on the surprising discovery that an existing commercial vaccine product is remarkably effective in preventing the disease. This vaccine is marketed under the name ~~"Renogen™" and comprises a live, non-virulent strain of *Arthrobacter*~~ RENOGEN, a live, non-virulent strain of *Arthrobacter* vaccine. Currently, this vaccine is indicated to protect salmon and other farmed fish against bacterial kidney disease (BKD). The characteristics of this strain are disclosed in WO 98/33884, which is incorporated herein by reference.

Please amend the following paragraphs that begin at the bottom of page 2 and continue on page 3 as follows:

~~The Renogen™~~ RENOGEN vaccine has been in use for some time to combat Bacterial Kidney Disease (BKD) in salmonid fish. This vaccine is unique in that it is the first live culture to have been licensed for use in aquaculture, and comprises a live culture of *Arthrobacter sp. nov.*, deposited under Accession No ATCC 55921 with the American Type Culture Collection (10801 University Boulevard, Manassas, VA 20110-2209) on 20 December 1996. *Arthrobacter* is not pathogenic to fish; nor is it the causative agent of BKD (which is *Renibacterium salmoninarum*).

It was observed on one site in the field that use of ~~Renogen™~~ RENOGEN in a salmon population at risk of contracting BKD led to a dramatic reduction in mortality rates compared to untreated fish. Average weight gain in the ~~Renogen-treated~~ RENOGEN-treated group was 18% greater than in the untreated fish group. SRS was also common on the site, which led the present inventors to speculate that ~~Renogen™~~ RENOGEN may have conferred hidden protection against SRS as well as BKD.

In order to test this concept, tank-held fish were immunized with ~~Renogen™~~ RENOGEN and subsequently challenged with *P. salmonis*, as described in Example 2. In the

negative control group, which had received saline injections, nearly all the fish succumbed to SRS. The test groups that had received the ~~Renogen™~~ RENOGEN vaccine exhibited extremely low mortality rates after 471 dd (degree days), amounting to between 88 and 100 relative percent survival (RPS). Even after 1441 dd (equivalent to one year in sea water) the test groups had a RPS of between 69 and 85%, compared to only 48.6% in the inactivated *P. salmonis* "gold standard" group.

Further evidence of the potential for vaccination with ~~Renogen™~~ RENOGEN is demonstrated by the cross-reactivity of *P. salmonis* antigen when probed with rabbit polyclonal anti-*Arthrobacter* antibodies (Example 1).

We have shown that ~~Renogen™~~ RENOGEN is more effective than any other known vaccine in preventing SRS. Live *Arthrobacter* bacteria are known to be able to enter cells and replicate for a limited period of time. The present inventors believe that this permits the antigen processing of both carbohydrate and protein antigens with sufficient homology to T-cell epitopes of *P. salmonis* to provide a high level of protection to direct challenge with virulent *P. salmonis*.

Please amend page 4, lines 7 and 8 as follows:

~~Renogen™~~ RENOGEN is based on a particular deposited strain of *Arthrobacter* (ATCC 59921). In performing the present invention, this strain or equivalent *Arthrobacter* strains can be

Please amend page 6, lines 7 through 28 as follows:

In one embodiment the *Arthrobacter* vaccine of the invention comprises an immunostimulant. The immunostimulant may be any known immunostimulant, but it is preferably a killed bacterial preparation. Preferably the immunostimulant is killed *Arthrobacter* cell material, which is optionally heat killed and is optionally from a culture of *Arthrobacter* ATCC 59921. Suitable examples of killed bacterial preparations include: ~~"Peptimune"~~ PEPTIMUNE (a heat-killed *Arthrobacter* ATCC 59921 culture) and ~~"Ultracorn"~~ ULTRACORN (ultrasonicated *Corynebacterium cutis* lysate). An optimal dosage of killed bacterial immunostimulant is (per vaccine unit dose) 1 to 100 µg, preferably in the range 5 to 50 µg, more preferably 10 to 20 µg and optionally about 12 µg of cellular matter. The killed bacterial immunostimulant is optionally dissolved or suspended in sterile diluent (e.g. saline) for mixing with lyophilized live *Arthrobacter* cells.

The invention in one aspect provides a vaccine composition comprising live *Arthrobacter* cells and further comprising at least one other immunogen (where an "immunogen" is defined as a molecule such as an antigen capable of raising a specific immune response in a fish). The immunogen is optionally selected from the group consisting of: inactivated antigen prepared from *Piscirickettsia salmonis (P. salmonis)*; a recombinant *P. salmonis* antigen; and a nucleic acid vector carrying an expressible *P. salmonis* antigen. In some instances it may be desirable to combine the ~~Renogen™~~ RENOGEN vaccine of the invention with a conventional SRS vaccine (*P.salmonis* bacterin or recombinant antigen vaccine or nucleic acid vaccine) in a kit comprising both components for separate, sequential or simultaneous administration, for treatment or prevention of SRS.

Please amend the paragraph at the bottom of page 8 and top of page 9 as follows:

Coho salmon (n=110 per treatment group, mean weight 10 g) were maintained under normal husbandry conditions in tank water according to standard operating procedures at 12 °C. Following one week of acclimatization Groups 1, 2 and 3 were vaccinated intraperitoneally with 0.1ml of 10^5, 10^6, and 10^7 cfu/dose, respectively, of lyophilized *Arthrobacter sp. nov* cells ~~(Renogen™)~~ (RENOGEN) reconstituted in saline diluent. Groups 4 and 5 were treated in an identical manner to Group 1, but with the addition of 12.2 µg and 50 µg per dose, respectively, of ~~"Peptimune"~~ PEPTIMUNE in the saline diluent. ~~Peptimune is a preparation of heat-killed *Arthrobacter* grown in liquid culture (MTSB broth) to a cell density of >1xE9, and standardized by protein assay to administer 12 and 50 µg per dose.~~ Groups 6 and 7 were positive controls vaccinated with *P. salmonis* bacterin. The bacterin was prepared from the supernatant of a *P. salmonis* type strain LF-89 infected CHSE-14 cell culture using 0.125% formalin at 4°C over a minimum 72h period. U/F concentration was employed and the concentrated supernatant was used to incorporate 8 µg (protein) per 0.1ml dose. The bacterin vaccine was delivered with ~~Ultracorn~~ ULTRACORN (Virbac, France) at 20 (Group 6) and 100 µg (Group 7) per fish. ~~Ultracorn is an immune stimulant based on an ultrasonicated *Corynebacterium cutis* lysate.~~ The antigens were emulsified with an equal volume of mineral oil adjuvant prior to injection. The negative control group (Group 8) received an injection of saline.

Please amend from the middle of page 15 through page 14 as follows:

~~Table 1:~~ Table 2: Mortality during the 28 d safety test, maintained at 9-12 °C through-out the safety and pre-challenge period.

Group	Treatment	Tank	Loss per treatment (N)	Total (N)	% Mortality
1	~~Renogen™~~ RENOGEN 10^5 dose	I1	0	110	0
2	~~Renogen™~~ RENOGEN 10^6 dose	I2	0	110	0
3	~~Renogen™~~ RENOGEN 10^7 dose	I3	7	110	6.3
4	~~Renogen™~~ RENOGEN 10^5 dose +12.2 µg ~~Peptimune~~ PEPTIMUNE	I4	1	110	0.9
5	~~Renogen™~~ RENOGEN 10^5 dose +50 µg ~~Peptimune~~ PEPTIMUNE	I5	4	110	3.6
6	*P. salmonis* 20U/Oil	I6	0	110	0
7	*P. salmonis* 100U/Oil	I7	0	110	0
8	Saline	I8	0	110	0

During the safety study, it was observed that fish in Group 3 suffered some loss (6.3%) nearing the end of the 28 d safety period. The lab investigator treated all fish in the population with a three day formalin treatment for bacterial gill disease. Mortality (3.6%) in Group 5 was recorded during the initial three day period pv, indicating that the inclusion of ~~Peptimune~~ PEPTIMUNE as 40% of the diluent was somewhat toxic. No positive plates were cultured from the losses during the safety period, either for the live vaccine strain, or any incidental bacterial cultures.

~~Table 2:~~ Table 3: Cumulative Mortality and Relative Percent Survival of Coho salmon (mean weight 10 g) 471 dd post-vaccination with *Arthrobacter sp. nov* cells (Groups 1-5), Inactivated SRS vaccines, or saline when challenged with virulent *P. salmonis* by intraperitoneal injection ($TCID_{50}$ 3 x 102.9 per fish) at 12 °C.

Group	Treatment	Tank	Loss per duplicate tank (N)	Total	Loss per treatment	% Mort	RPS
1	~~Renogen™~~ RENOGEN 10^5 dose	L1, L2	0/25, 1/25	50	1/50	2	97.6
2	~~Renogen™~~ RENOGEN 10^6 dose	L3, L4	1/26, 0/24	50	1/50	2	97.6
3	~~Renogen™~~ RENOGEN 10^7 dose	L5, L6	2/25, 3/25	50	5/50	10	88.1
4	~~Renogen™~~ RENOGEN 10^5 dose +12.2 µg ~~Peptimune~~ PEPTIMUNE	L7, L8	0/25, 0/25	50	0/50	0	100
5	~~Renogen™~~ RENOGEN 10^5 dose +50 µg ~~Peptimune~~ PEPTIMUNE	L9, L10	0/25, 0/25	50	0/50	0	100
6	*P. salmonis* 20U/Oil	L11, L12	9/25, 12/25	50	21/50	42	50.0
7	*P. salmonis* 100U/Oil	L13, L14	7/25, 6/25	50	13/50	26	69.1
8	Saline	L15, L16	19/25, 23/25	50	42/50	84	----

At 471 dd post-vaccination, fish in Group 1 had a relative percent survival (RPS) of 97.6, a high level of protection from direct infection with *P. salmonis* over 32 days, where mortality in the saline control group was 84%. This compared favourably to the protection garnered from vaccination with the standard inactivated vaccines (Groups 6 and 7), that showed RPS values of 50 and 69% respectively.

$TCID_{50}$ Analysis of Surviving Fish in Group 1, 7 and 8.

Table 4: Level of SRS infection in the tissue samples of the surviving fish from the 471 dd challenge (n=7-10), 32 days post-infection :

Group	Treatment	% of fish $TCID_{50}$ $>10^2$/mL	Mean $TCID_{50}$
1	~~Renogen~~™ RENOGEN	20	104.5/mL
7	*P. salmonis* 100U/oil	44	104.6/mL
8	Saline	50	104.7/mL

The $TCID_{50}$ of the fish sampled from the ~~Renogen~~™ RENOGEN group was lower than the inactivated vaccine group, and both were lower than the saline controls. This is not of apparent clinical relevance, as the contribution of the high titre groups negates the lower infective dosages when averaging. However, the ~~Renogen~~™ RENOGEN group did have the lowest percent positives (<20%) as samples with less than 10^2 were considered not to be clinically infected with SRS. This compares to the same samples from the saline control group where 50% of the fish were positive for SRS, and favourably to the inactivated vaccine group with 44% of the fish positive for SRS.

~~Table 3:~~ Table 5: Cumulative Mortality and Relative Percent Survival of Coho salmon (mean weight 10 g) 1441 dd post-vaccination with *Arthrobacter sp. nov* cells (Groups 1-5), Inactivated SRS vaccines, or saline when challenged with virulent *P. salmonis* by intraperitoneal injection (TCID 3 x 102.9 per fish) at 12 °C.

Group	Treatment	Tank	Loss per duplicate tank (N)	Total	Loss per treatment	% Mort	RPS
1	~~Renogen™~~ RENOGEN 10^5 dose	L1, L2	8/25, 3/25	50	11/50	22	69.4
2	~~Renogen™~~ RENOGEN 10^6 dose	L3, L4	2/24, 3/25	49	5/49	10.2	85.8
3	~~Renogen™~~ RENOGEN 10^7 dose	L5, L6	3/19, 2/19	38	5/38	13.2	81.7
4	~~Renogen™~~ RENOGEN 10^5 dose +12.2 µg ~~Peptimune~~ PEPTIMUNE	L7, L8	4/25, 5/25	52	9/52	17.2	76.1
5	~~Renogen™~~ RENOGEN 10^5 dose +50 µg ~~Peptimune~~ PEPTIMUNE	L9, L10	2/24, 5/24	48	7/48	14.6	79.7
7	*P. salmonis* 100U/Oil	L11, L12	10/23, 7/23	46	17/43	37	48.6
8	Saline	L13, L14	20/25, 16/25	50	36/50	72	----

Note: back-up fish in Group 6 intended for the long term efficacy study were lost due to accidental shut-off of water flow in this tank (I7).

After an elapsed period of 1140 dd, the durational response of the protection observed at the earlier test period (471 dd) was assessed. Results of the second challenge where a level of 72% mortality was observed in the saline control group indicate that the level of protection is still high with ~~Renogen~~™ RENOGEN treated fish (69.4% RPS), with some indication that a higher dosage may improve the long term protection (10^6 and 10^7 cfu/dose had RPS of 85.8 and 81.7 respectively). The addition of the immunostimulant ~~Peptimune~~ PEPTIMUNE at 12 and 50 µg to the diluent provided an improvement to the efficacy of the product at dose (76.1 and 79.7 % respectively). The accidental loss of the standard reference vaccine (group 6) allowed for comparison to Group 7 only, and this group had an RPS of 48.6%.

CONCLUSIONS:

~~Renogen~~ RENOGEN provided significant protection against direct challenge with *P. salmonis* at 471 dd and at 1441 dd post-vaccination. The vaccine was superior to the protection provided by the standard oil vaccine. We were able to demonstrate that fewer surviving fish in the ~~Renogen~~™ RENOGEN group were clinically infected with *P. salmonis*. The study demonstrates that *Arthrobacter sp. nov.* live vaccine provides a high degree of protection against *P. salmonis* infection, and that the protective effect is shown to be long-term. Inclusion of a killed *Arthrobacter* preparation in the vaccine had an immune-stimulating effect resulting in improved survival rates.